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January 19, 2005

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APPLICATION NUMBER: *60/552,528*
FILING DATE: *March 12, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US04/40674*

Certified by

Jon W. Dudas

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ATTENTION: PROVISIONAL PATENT APPLICATION

Sir:

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c).

For: **REPERTOIRE CLONING OF CHIMPANZEE FAB FRAGMENTS AND PRODUCTION OF FULL-LENGTH HUMANIZED IGG1 ANTIBODIES EFFICIENT FOR NEUTRALIZATION OF DENGUE TYPE 1-4 VIRUSES**

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Enclosed are:

(X) Specification in 2 pages.

(X) A check in the amount of $160 to cover the filing fee is enclosed.

(X) A return prepaid postcard.

(X) The Commissioner is hereby authorized to charge any additional fees which may be required, now or in the future, or credit any overpayment to Account No. 11-1410.

Was this invention made by an agency of the United States Government or under a contract with an agency of the United States Government?

(X) Yes. The name of the U.S. Government agency and the Government contract number are: National Institutes of Health.

(X) Please send correspondence to:

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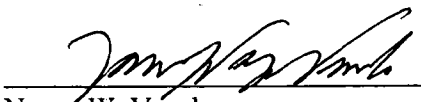
Respectfully submitted,



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CERTIFICATE OF MAILING BY "EXPRESS MAIL"

Attorney Docket No.	:	NIH272.003PR
Applicant(s)	:	Lai et al.
For	:	REPERTOIRE CLONING OF CHIMPANZEE FAB FRAGMENTS AND PRODUCTION OF FULL-LENGTH HUMANIZED IGG1 ANTIBODIES EFFICIENT FOR NEUTRALIZATION OF DENGUE TYPE 1-4 VIRUSES
Attorney	:	Nancy W. Vensko
"Express Mail" Mailing Label No.	:	EV 309081509 US
Date of Deposit	:	March 12, 2004

I hereby certify that the accompanying

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are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and are addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Victoria Roeser

Victoria Roeser

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Repertoire Cloning of Chimpanzee Fab Fragments and Production of Full-Length Humanized IgG1 Antibodies Efficient for Neutralization of Dengue Type 1-4 Viruses.

Passive immunization using monoclonal antibodies from humans or non-human primates represents an attractive alternative for prevention of dengue. Chimpanzees were inoculated with infectious dengue type 4 virus (DENV-4) RNA intra-hepatically and then with a mixture of the other three dengue serotype viruses nine months later. Fab antibody fragments reactive to each of the four dengue serotype viruses were recovered by repertoire cloning of bone marrow mRNA from one of the chimpanzees that developed higher antibody titers. These Fab monoclonal antibodies were analyzed for antigen binding specificity, V_H and V_L sequences, and neutralizing activity against each dengue virus by plaque reduction neutralization test (PRNT). Serotype specific Fabs that neutralized DENV-4 and cross-reactive Fabs that neutralized both DENV-1 and DENV-2 at a high titer were identified. The dengue serotype cross-reactive Fab antibodies also neutralized DENV-3, DENV-4 or other members of the flaviviruses, including the West Nile virus at a reduced titer. Several of these Fabs were converted to the full-length IgG1 antibodies in combination with the human sequences. Humanized antibody IgG1 5H2 neutralized DENV-4 from different geographical origins at a similar $PRNT_{50}$ titer of 0.03-0.05 μg/ml. Humanized antibody IgG1 1A5 also neutralized DENV-1 and DENV-2 at a high titer. These humanized monoclonal antibodies may prove valuable for passive immunization against dengue in humans.

Epitope Determinants of a Chimpanzee Fab Antibody that Cross-Neutralized Dengue 1 and Dengue 2 Viruses Mapped in the Fusion Peptide Loop of the Envelope Protein.

The epitope determinants of a chimpanzee monoclonal antibody, Fab 1A5, that had been shown to be broadly cross-reactive to flaviviruses and efficient for neutralization of both dengue 1 and dengue 2 viruses at a similar titer, were studied by analysis of dengue 2 antigenic variants. Sequence analysis showed that one antigenic variant contained a Gly toVal substitution at position 106 within the flavivirus-conserved fusion peptide loop in the envelope protein (E) and another antigenic variant contained a His to Gln substitution at position 317 in E. Substitution of $Gly_{106}Val$ reduced Fab 1A5 binding by approximately 80 fold, whereas substitution of $His_{317}Gln$ had little or no effect on antibody binding as compared to the parental virus. In an ELISA, binding of Fab 1A5 to the dengue 2 virus was competed by an oligopeptide containing the fusion peptide sequence. Fab 1A5 inhibited low pH-induced membrane fusion of dengue 1- or dengue 2-infected mosquito C6/36 cells as demonstrated by reduced syncytium formation. The result from fusion-from-within assay showed that both substitutions in E of the dengue 2 variants lowered the pH threshold for membrane fusion of the infected C6/36 cells. In the 3-D E structure, Gly106 in domain II and His317 in domain III of the opposite E monomer are spatially close. From the locations of these amino acids, monoclonal antibody Fab 1A5 appears to recognize a novel epitope that has not been mapped before for mouse monoclonal antibodies.